REMARKS/ARGUMENTS

Claims 28-35 and 38-47 are pending in the instant application.

I. Claim Rejections Under 35 U.S.C. §101 and §112, First Paragraph

Claims 28-35 and 38-47 remain rejected under U.S.C. §101 allegedly "because the claimed invention in not supported by either a credible, specific and substantial asserted utility or a well established utility." (Page 2 of the instant Final Office Action).

Claims 28-35 and 38-47 remain further rejected under 35 U.S.C. §112, first paragraph, allegedly "since the claimed invention in not supported by either a credible, specific and substantial asserted utility or a well established utility for the reasons set forth above, one skilled in the art clearly would not know how to use the claimed invention." (Page 2 of the instant Final Office Action).

Applicants submit, as discussed below, that not only has the PTO not established a *prima facie* case for lack of utility, but that the polypeptides of Claims 28-35 and 38-47 possess at least one specific and substantial asserted utility, and that based upon this utility, one of skill in the art would know how to use the claimed polypeptides without undue experimentation.

The results of the adipocyte glucose/FFA uptake assay provide utility for the PRO1303 polypeptide

One patentable utility of the claimed PRO1303 polypeptides is based upon the results of the adipocyte glucose/FFA uptake assay for this polypeptide. The specification discloses that the adipocyte glucose/FFA uptake assay is designed to determine whether a polypeptide is capable of modulating, either positively or negatively, the uptake of glucose or free fatty acids in adipocyte cells. By making such determinations, the assay identifies polypeptides that are expected to be useful for treating disorders wherein stimulation or inhibition of glucose uptake by adipocytes is expected to be therapeutically effective, for example, diabetes, and hyper- or hypo-insulinemia. PRO1303 resulted in more than 1.5 times the uptake of the insulin control, and therefore PRO1303 tested positive as a stimulator of glucose/FFA uptake in adipocyte cells.

Accordingly, Applicants respectfully submit that at the effective filing date of the instant application, one of skill in the art would have reasonably accepted that various compounds, such as PRO1303, that are capable of modulating glucose uptake have a substantial, practical, real life utility. The above-mentioned studies have clearly established that the glucose/FFA uptake assay

as described in the instant application is a reliable assay system to identify therapeutic agents for treating diseases and conditions such as obesity, diabetes, and hyperinsulinemia. Therefore, Applicants respectfully submit that a variety of real-life utilities, such as treatments for glucose uptake related diseases, including obesity and diabetes, are envisioned for PRO1303 based on the glucose/FFA uptake assay results disclosed herein.

The Examiner maintains that "the specification does not indicate which asserted utilities correspond specifically to glucose uptake stimulation as opposed to glucose uptake inhibition." The Examiner continues to assert that "the specification does not indicate what, if any, of the utilities set forth correspond to stimulation of FFA uptake." The Examiner further asserts that stimulation of glucose and/or FFA uptake is actually "three very different activities (stimulation of glucose uptake only, stimulation of FFA uptake only, and stimulation of uptake of both)." Finally, the Examiner asserts that "it is unclear how increasing uptake of FFA into adipocytes would treat obesity (or thus diabetes)." In support of these assertions, the Examiner cites Fabris et al. and Santomauro et al.(Pages 2-4 of the instant Final Office Action).

Applicants maintain, for the reasons provided in the previously filed response of November 13 2006 and Appeal Brief of April 24, 2006, that one of ordinary skill in the art would find it more likely than not that an agent which increases uptake of glucose and/or FFA by adipocytes would also be useful in the treatment of disorders such as diabetes, hyperglycemia, and obesity. Accordingly, a *prima facie* showing of lack of utility has not been made in this instance and the burden to provide further evidence of utility has not shifted to Applicants.

The glucose/FFA uptake assay, as described in Example 149 of the instant application, was well known in the art at the time of the effective filing date of the instant application. As demonstrated by the references of record, similar assays were commonly used to identify potential anti-diabetic agents. For instance, at the time of the effective filing date of the instant application, it was well known in the art that increasing glucose uptake by adipocyte cells is a hallmark of a number of therapeutically effective agents, such as troglitazone and pioglitazone. Treatment with vanadium salts, another agent which increased glucose uptake, was shown to lower glucose levels in hyperglycemic rats. Diabetes, hyperglycemia, and obesity were know at the time of filing to be closely linked conditions (see, for example, Sandouk, page 352). Thus, the art has shown that agents which decrease circulating FFA levels (or increase glucose/FFA

uptake by adipocytes) are useful in the treatment of disorders such as diabetes, hyperglycemia, and obesity.

Applicants also submit herewith references by Liu et al. (J. Nutr. 2005, 135(2), pp. 165-171), Gowri et al. (Metabolism, 1999, 48(4), pp. 411-414) and Edens et al. (J. Nutr. 2002, 132(6), pp. 1141-1148) as further support that stimulation of glucose uptake by adipocytes is a fairly common way of testing pharmaceutical agents for treating diabetes. Edens et al. teach that food components that enhance glucose uptake and metabolism may act as "insulin-mimetic" agents, which work in the absence of insulin, or as "insulin-potentiating agents" which enhance the action of insulin but have no independent effects on glucose metabolism. Liu et al. used glucose uptake assays as a way to measure activities that stimulated glucose transport in efforts to characterize the properties of tannic acid and from these experiments concluded that tannic acid may be useful for the prevention and treatment of type II diabetes and its associated obesity. Finally, Gowri et al. teach that masoprocol stimulates glucose uptake and inhibits isoproterenol-induced lipolysis when incubated with rat adipocytes and conclude that these findings provide a mechanistic explanation at the cellular level to account for the decrease in serum glucose, triglyceride, and FFA concentration observed in masoprocol-treated rodent models of type 2 diabetes.

Applicants note that the evidentiary standard to be used throughout *ex parte* examination in setting forth a rejection is a preponderance of the totality of the evidence under consideration. Thus, to overcome the presumption of truth that an assertion of utility by the applicant enjoys, the Examiner must establish that it is more likely than not that one of ordinary skill in the art would doubt the truth of the statement of utility. Only after the Examiner has made a proper *prima facie* showing of lack of utility, does the burden of rebuttal shift to the applicant. Therefore, the legal standard for patentable utility is not absolute certainty. Clear evidence supports the glucose/FFA uptake enhancing activity of PRO1303. On the other hand, the Examiner has provided no evidence to demonstrate that it more likely than not that one of skill in the art would doubt the truth of this asserted utility of PRO1303 as an enhancer of glucose/FFA uptake.

It is known in the art that FFA levels regulate glucose uptake by adipocytes. Thus, even if the actual mechanistic effect of PRO1303 is only to directly increase FFA uptake by adipocyte cells, this will necessarily result in indirectly increasing glucose uptake by adipocytes.

Furthermore, agents which are well known in the art as useful in the treatment of diabetes, such as the thiazolidenediones, have been shown to exert their effects, at least in part, through the increase of FFA uptake by adipocytes. Accordingly, an agent which increases FFA uptake by adipocytes has the same utility in the treatment of disease as those recognized by the Examiner for agents which enhance glucose uptake. Thus, the polypeptide of SEQ ID NO: 194 has a significant and presently available benefit to the public as a peptide useful for diabetes research. The Examiner has not presented evidence specific to the claimed polypeptide to refute that.

Accordingly, Applicants submit that when the proper legal standard is applied, one should reach the conclusion that the present application discloses at least one patentable utility for the claimed PRO1303 polypeptides. Further, based on this utility, one of skill in the art would know exactly how to use the claimed polypeptides without any undue experimentation.

The gene amplification data disclosed in Example 143 establishes a credible, substantial and specific patentable utility for the PRO1303 polypeptides.

Applicants respectfully note that they are required to disclose only a single patentable utility for their claimed invention. Nonetheless, in addition to the utilities discussed above in the treatment of disorders such as obesity, diabetes, and hyper- or hypo-insulinemia, Applicants respectfully submit that the gene amplification data also demonstrates patentable utility for the PRO1303 polypeptide. The gene amplification data for the gene encoding the PRO1303 polypeptide is clearly disclosed in the instant specification under Example 143. Applicants respectfully maintain the position that the specification discloses at least one credible, substantial and specific asserted utility for the claimed PRO1303 polypeptides for the reasons previously set forth in Applicants' Response of November 13, 2006 and Appeal Brief filed April 24, 2006.

As discussed in Applicants' previous Responses and Appeal Brief, the specification discloses that the nucleic acids encoding PRO1303 had ΔCt value of > 1.0, which is a **more than 2-fold increase**, for primary lung tumors LT13, LT15, LT16; for lung cell line A549; and for the primary colon tumor CT16. PRO1303 showed approximately 1.13 to 1.42 ΔCt units which corresponds to $2^{1.13}$ to $2^{1.42}$- fold amplification or 2.19 to 2.68 fold amplification in primary lung tumors LT13, LT15, LT16; for lung cell line A549; and for the primary colon tumor CT16. (See Table 8 of the specification). Accordingly, the present specification clearly discloses strong evidence that the gene encoding the PRO1303 polypeptide is significantly amplified in a number

of lung and colon tumors. Thus one of ordinary skill in the art would find it credible that the claimed PRO1303 polypeptides have utility as diagnostic markers of lung and colon tumors.

A prima facie case of lack of utility has not been established

The Examiner asserts that the "[t]he evidence of record indicates that (1) gene amplification does not reliable correlate with increased mRNA levels (Pennica et al., Konopka et al.), (2) increased mRNA levels do not reliably correlate with increased polypeptide levels in the majority of cases (Haynes et al., Gygi et al., Lian et al., Fessler et al., Hu et al., LaBaer et al., Chen et al., Hanna et al.), (3) no evidence has been brought forth regarding levels of PRO1303 mRNA levels or PRO1303 polypeptide levels in cancerous tissue." (Pages 11-12 and 18 of the instant Final Office Action).

Applicants also maintain, for the reasons provided in the previously filed responses, that Pennica *et al.,* Konopka *et al.*, Haynes *et a*l., Gygi *et al.*, Lian et al., Fessler et al., Hu *et al.*, LaBaer *et al.*, Chen *et al.* and Hanna *et al.* do not show that a lack of correlation between gene (DNA) amplification and elevated mRNA levels, in general, exists. Applicants' arguments presented in the previously filed Response of November 13, 2006 and previous responses of record are hereby incorporated by reference in their entirety.

As discussed in Applicants' Brief filed April 24, 2006, the evidentiary standard to be used throughout *ex parte* examination of a patent application is a preponderance of the totality of the evidence under consideration. Accordingly, Applicants submit that in order to overcome the presumption of truth that an assertion of utility by the applicant enjoys, the Examiner must establish that **it is more likely than not** that one of ordinary skill in the art would doubt the truth of the statement of utility. **The standard is not absolute certainty.** The law requires only that one skilled in the art should accept that such a correlation is **more likely than not to exist**. Applicants submit that the references cited by the PTO are either irrelevant, or actually offer support for Applicants' position, as discussed below. Even if the PTO has met its initial burden, Applicants have submitted enough rebuttal evidence such that it is **more likely than not** that a person of skill in the art would be convinced, **to a reasonable probability,** that the asserted utility is true.

Pennica *et al.* and Konopka *et al.*

In response to Applicants' previous arguments, the Examiner argues that "Pennica et al and Konopka et al. are relevant even though they are not reviews of gene amplification for genes in general because they show a lack of correlation between gene amplification and gene product overexpression" and because the instant case also concerns a single gene. (Page 11 of the instant Final Office Action).

Applicants respectfully disagree. The test is whether it is more likely than not that gene amplification results in overexpression of the corresponding mRNA and protein. In order to meet that standard, the Examiner must provide evidence that it is more likely than not that gene amplification does not result in mRNA or protein overexpression. Providing the single example of the WISP-2 gene does not suffice to meet this burden.

Applicants next respectfully submit that, contrary to the PTO's assertions, Konopka *et al.* **supports** Applicants' position that mRNA levels correlate with protein levels. Konopka *et al.* states that "the 8-kb mRNA that encodes $P210^{c\text{-}abl}$ was detected at a 10-fold higher level in SK-CML7bt-333 (Fig. 3A, +) than in SK-CML16Bt-1 (B, +), which **correlated** with the relative level of $P210^{c\text{-}abl}$ detected in each cell line. Analysis of additional cell lines demonstrated that the level of 8-kb mRNA **directly correlated** with the level of $P210^{c\text{-}abl}$ (Table 1)" (page 4050, col. 2, emphasis added).

Nor does Konopka *et al.* support the PTO's position that DNA amplification is not correlated with mRNA or protein overexpression. Konopka *et al.* show only that, of the cell lines known to have increased abl protein expression, only one had amplification of the abl gene (page 4051, col. 1). This result proves only that increased mRNA and protein expression levels can result from causes other than gene amplification. Konopka *et al.* do not demonstrate that when gene amplification does occur, it does not result in increased mRNA and protein expression levels, particularly given that the cell line with amplification of the abl gene did show increased abl mRNA and protein expression levels.

Haynes *et al.*

The Examiner maintains that the Haynes et al. reference establishes that "protein expression levels are not predictable from the mRNA expression levels...and only the direct analysis of mature protein products can reveal their correct identities, their relevant state of

modification and/or association and their amounts." (Page 12 of the instant Final Office Action).

As a preliminary matter, it is not a legal requirement to establish a "necessary" correlation between an increase in the copy number of the mRNA and protein expression levels that would correlate to the disease state or that it is "imperative" to find evidence that protein levels can be accurately predicted. As discussed above, the evidentiary standard to be used throughout *ex parte* examination of a patent application is a preponderance of the totality of the evidence under consideration. Accordingly, the question is not whether a necessary or even "strong" correlation between an increase in copy number and protein expression levels exists, rather if it is more likely than not that a person of ordinary skill in the pertinent art would recognize such a positive correlation. Applicants respectfully submit that when the proper evidentiary standard is applied, a correlation must be acknowledged.

Indeed, contrary to the Examiner's reading, Haynes teaches that "there was a *general trend but no strong correlation* between protein [expression] and transcript levels." (Emphasis added). For example, in Figure 1, there is a positive correlation between mRNA and protein levels amongst most of the 80 yeast proteins studied. In fact, very few data points deviated or scattered away from the expected normal and no data points showed a negative correlation between mRNA and protein levels (*i.e.,* an increase in mRNA resulted in a decrease in protein levels). The analysis by Haynes *et al.* is not relevant to the current application. Haynes was studying yeast cells and not human cells. Haynes *et al* notes that their analysis focused on the 80 most abundant proteins in the yeast lysate. (Page 1867). Haynes *et al.* states "since many important regulatory protein are present only at low abundance, these would not be amenable to analysis." (Page 1867). Further, Haynes *et al.* compared the protein expression levels of these naturally abundant proteins to mRNA expression levels from published SAGE frequency tables. (Page 1863). Accordingly, Haynes *et al.* did not compare mRNA expression levels and protein levels in the same yeast cells. Thus, the analysis by Haynes *et al.* is not applicable to the present application.

Hu *et al.*

Applicants have already analyzed the teaching of Hu in the previously submitted Preliminary Amendment of November 13, 2006. Applicants have submitted that Hu does not

teach a lack of correlation between mRNA and protein expression. Applicants maintain the same position. Applicants also repeat that Hu *et al.* did not look for a correlation between changes in mRNA and changes in protein levels, and therefore their results are not contrary to Applicants' assertion that there is a correlation between the two. Applicants are not relying on any "biological role" that the PRO1303 polypeptide has in cancer for its asserted utility. Instead, Applicants are relying on the overexpression of PRO1303 in certain tumors compared to their normal tissue counterparts. Nowhere in Hu does it say that a lack of correlation in their study means that genes with a less than five-fold change in level of expression in cancer cannot serve as a diagnostic marker of cancer.. Thus, the Examiner has provided no reason why the various thresholds (such as 5 fold, 10 fold threshold) determined by Hu *et al.* for indicating whether a gene plays a biological role in one specific type of breast cancer are relevant to determining whether a gene is useful as a diagnostic marker for all types of breast cancer, let alone for lung or colon cancer.

The Examiner further asserts that "Applicant is holding Hu *et al.* to a higher standard than their own specification" for statistical analysis. (Page 14 of the instant Final Office Action). However, Applicants have compared the level of amplification of the PRO1303 gene in normal tissue and lung and colon tumors and have provided information indicating a greater than 2-fold amplification. Applicants are not relying on statistical analysis of information obtained from published literature based on the current research interest of a molecule, and hence the issues regarding statistical analysis of such information do not apply to Applicants' data.

Hanna *et al.*

The Examiner asserts that "Hanna et al. show that gene amplification does not reliably correlate with polypeptide over-expression, and thus the level of polypeptide expression must be tested empirically." (Page 13 of the instant Final Office Action).

Applicants respectfully point out that the Examiner appears to have misread Hanna *et al.* Hanna *et al.* clearly state that gene amplification (as measured by FISH) and polypeptide expression (as measured by immunohistochemistry, IHC) are well correlated ("in general, FISH and IHC results correlate well" (Hanna *et al.* p. 1, col. 2)). It is only a subset of tumors which show discordant results. Thus Hanna *et al.* support Applicants' position that it is more likely than not that gene amplification correlates with increased polypeptide expression.

Applicants have clearly shown that the gene encoding the PRO1303 polypeptide is amplified in a number lung and colon tumors and cell lines. Therefore, the PRO1303 gene, similar to the HER-2/neu gene disclosed in Hanna *et al.*, is a tumor associated gene. Furthermore, as discussed above, in the majority of amplified genes, the teachings in the art overwhelmingly show that gene amplification influences gene expression at the mRNA and protein levels. Therefore, one of skill in the art would reasonably expect in this instance, based on the amplification data for the PRO1303 gene, that the PRO1303 polypeptide is concomitantly overexpressed.

Chen *et al.*

The Examiner again cites Chen et al. as allegedly disclosing that "only 17% of 165 polypeptide spots or 21% of the genes had a significant correlation between protein and mRNA expression levels" in lung adenocarcinoma samples. (Page 14 of the instant Final Office Action).

Applicants reiterate that, as discussed in their previous Responses, no attempt was made to compare expression levels in normal versus tumor samples, and in fact the authors concede that they had too few normal samples for meaningful analysis (page 310, col. 2). As a result, the analysis in the Chen paper shows only that a number of randomly selected proteins have varying degrees of correlation between mRNA and protein expression levels within a set of different lung adenocarcinoma samples. The Chen paper does not address the issue of whether increased mRNA levels in the tumor samples taken together as one group, as compared to the normal samples as a group, correlated with increased protein levels in tumorous versus normal tissue.

Applicants have asserted that an increase in mRNA expression in tumor tissue as compared to normal tissue will, in general, correlate with increased protein expression in the same tumor tissue as compared to normal tissue. Chen *et al.* did not examine the correlation between increases in mRNA and protein expression in tumor tissue as compared to normal tissue and says nothing about it. Accordingly, the results presented in the Chen paper are not applicable to the application at issue.

The Examiner further refers to the previously cited reference by Chen et al., asserting that "[w]hile 2D gels might exclude low abundance proteins, their use is valid for detectable proteins." (Page 16 of the instant Final Office Action).

Applicants submit that Kuo *et al.*, made of record the Response of November 13, 2006, explains that the problems with selecting proteins detectable by 2D gels, include that "most of the spots observed in the 2-D gels are isoforms of some proteins. **The intensity of each spot does not necessarily represent total amount of a certain protein** and thus does not correlate with it mRNA level" (page 904, col. 1; emphasis added). Thus, the issue with Chen *et al.*'s use of 2D gels is not simply that the method limits the proteins examined to a small and non-representative subset, but that the protein levels measured are not necessarily accurate, and that therefore correct conclusions regarding the correlation of mRNA levels to protein levels cannot be drawn.

The Examiner further asserts that Chen et al. "clearly answered the question posed: Does mRNA expression correlate with protein expression in lung tumor samples?." According to the Examiner, "the answer was 'no' in a majority of cases." (Page 17 of the instant Final Office Action).

Applicants respectfully submit that the Examiner appears to have misinterpreted the methodology of Chen *et al.* As explained by Applicants in their Response filed November 13, 2006, Chen *et al.* did not do a comparative assay in which expression levels from lung tumors were compared to those from a normal lung tissue control. Instead, the authors measured absolute expression levels in lung tumor samples, as well as a few normal lung samples. The expression levels were normalized relative to the median gene expression profile for the entire sample, not relative to a normal control (see page 306, col. 1). Thus the Chen paper does not address the issue of whether increased mRNA levels in the tumor samples taken together as one group, as compared to the normal samples as a group, correlated with increased protein levels in tumorous versus normal tissue. Accordingly, the results presented in the Chen paper are not applicable to the application at issue.

Lian *et al.* and Fessler *et al.*

The Examiner maintains that these previously cited references indicated a poor correlation between mRNA expression and protein abundance (Pages 8 and 15 of the instant Final Office Action).

Applicants respectfully reiterate the fact that in these papers, expression levels were only measured at all for many fewer proteins than transcripts. Since the expression levels of so many

fewer proteins than transcripts were measured, it is hardly surprising that a smaller absolute number of proteins than mRNAs were found to be overexpressed, because the protein products of most of the overexpressed mRNAs would not have been among the small number of proteins identified on the gels.

Furthermore, as admitted, for example, by Fessler *et al.,* protein identification by two-dimensional PAGE limited to well-resolved regions of the gel, may perform less well with hydrophobic and high molecular weight proteins, and tends to select for more abundant protein species (page 31301, col. 1). In addition, because protein binding of Coomassie Blue has a limited dynamic range and is typically not linear throughout the range of detection, image analysis of Coomassie Blue-stained protein spots should only be considered as semi-quantitative (see page 31301, col. 1). Thus those proteins whose expression levels are measured in these proteomics experiments are a small and unrepresentative subset of all proteins expressed in the studied cell type. Applicants point out that when efforts are made to accurately measure the expression levels of less abundant proteins, as in Futcher *et al.*, made of record November 13, 2006, the observed correlation between mRNA and protein expression levels is notably stronger.

Applicants next note that cases in which protein levels changed while mRNA levels were unchanged are not relevant, since Applicants are not asserting that changes in mRNA levels are the only cause of changes in protein levels. Applicants are asserting that a measurable change in mRNA level generally leads to a corresponding change in the level of protein expression, not that changes in protein level can be used to predict changes in mRNA level. Thus Applicants do not dispute that measuring changes in protein levels adds useful information not found solely by measuring mRNA levels, because it is understood that not all changes in protein levels are the result of changes in RNA, but may be caused by later, translational regulatory events. This fact does not, however, affect Applicants' assertion that changes in mRNA levels in tumor as compared to normal tissue are generally predictive of changes in expression of the corresponding protein.

Applicants reiterate that they need only show that there is a correlation between mRNA and protein levels, such that mRNA overexpression generally predicts protein overexpression. A showing that mRNA levels can be used to "accurately predict" the precise levels of protein expression is not required.

Finally, the Examiner asserts that "the asserted utility that PRO1303 polypeptides and antibodies are useful as diagnostic markers for cancer is not substantial in that further research is required to reasonably confirm a real world context of use" (Page 18 of the instant Final Office Action)

Applicants respectfully disagree with the Examiner's assertion that the disclosed utility is not substantial. As indicated above, Applicants have provided evidence in the specification that the PRO1303 gene is amplified in lung and colon tumors. As previously discussed, the Examiner "must establish that it is more likely than not that a person of ordinary skill in the art would not consider that any utility asserted by the Applicants would be specific and substantial" in order to establish a *prima facie* case of lack of utility. M.P.E.P. §2107.02, Part IV. The Examiner has made no such showing. Applicants respectfully submit that it is more likely than not that a person of ordinary skill in the art would consider the asserted utility to be substantial based on the evidence provided in the specification, without needing to conduct any further research.

The M.P.E.P. provides the following guidelines for evaluating Applicants' evidence in support of an asserted utility:

> There is no predetermined amount or character of evidence that must be provided by an applicant to support an asserted utility, therapeutic or otherwise. Rather, the character and amount of evidence needed to support an asserted utility will vary depending on what is claimed, and whether the asserted utility appears to contravene established scientific principles and beliefs.... Furthermore, the applicant does not have to provide evidence sufficient to establish that an asserted utility is true "beyond a reasonable doubt."... Nor must an applicant provide evidence such that it establishes an asserted utility as a matter of statistical certainty.... Instead, evidence will be sufficient if, considered as a whole, it leads a person of ordinary skill in the art to conclude that the asserted utility is more likely than not true.

M.P.E.P. §2107.02, Part VII (internal citations omitted) (first and second emphases added, third emphasis in original). *See also Nelson v. Bowler*, 206 USPQ 881, 885 (CCPA 1980), cited in the M.P.E.P. at §2107.02, Part VII ("Relevant evidence is judged as a whole for its persuasiveness in linking observed properties to suggested uses. *Reasonable correlation* between the two is sufficient....") (Emphasis added). The evidence of record provide ample demonstration that there is in fact a "reasonable correlation" between changes in gene

amplification , mRNA levels and protein levels, as precisely such correlations have been observed in numerous references in the art.

It is "more likely than not" for increased mRNA levels to predict increased protein levels

Applicants have submitted ample evidence to show that, in general, if a gene is amplified in cancer, it is more likely than not that the encoded protein will be expressed at an elevated level. First, the articles by Orntoft *et al.*, Hyman *et al.*, and Pollack *et al.*, (of record in Information Disclosure Statement filed on June 29, 2005) and the articles by Bea *et al.* and Godbout *et al.* (of record in Information Disclosure Statement filed on November 13, 2006) collectively teach that in general, gene amplification increases mRNA expression.

Second, Applicants have submitted over a hundred references, along with the Declarations of Dr. Paul Polakis (Polakis II) and Dr. Randy Scott with their Response filed on November 13, 2006, which collectively teach that, in general, there is a correlation between mRNA levels and polypeptide levels. These declarations are being resubmitted with the instant response because the Examiner has indicated that neither declaration was received in this case. Consideration and entry of these Declarations is hereby requested.

Third, Applicants would like to bring to the Examiner's attention a recent decision by the Board of Patent Appeals and Interferences (Decision on Appeal No. 2006-1469). In its decision, the Board reversed the utility rejection, acknowledging that "there is a strong correlation between mRNA levels and protein expression, and the Examiner has not presented any evidence specific to the PRO1866 polypeptide to refute that." (Page 9 of the Decision). Applicants submit that, in the instant application, the Examiner has likewise not presented any evidence specific to the PRO1303 polypeptide to refute Applicants' assertion of a correlation between mRNA levels and protein expression.

Orntoft *et al.*, Hyman *et al.*, and Pollack *et al.*

With respect to Orntoft et al., who also looked at mRNA and protein expression levels of multiple genes, the Examiner asserts that Orntoft et al. "do not appear to look at gene amplification, mRNA levels and polypeptide levels from a single gene at a time." (Page 22 of the instant Final Office Action).

Applicants respectfully submit that, in addition to their analysis of gene amplification, Orntoft *et al.* also looked at the correlation between mRNA levels and protein expression levels for individual genes. Orntoft *et al.* clearly explain that "[i]n general **there was a highly significant correlation ($p<0.005$) between mRNA and protein alterations**. Only one gene [of the 40 examined] showed disagreement between transcript alteration and protein alteration" (page 42, col. 2; emphasis added). Clearly, a correlation in 39 of 40 genes examined supports Applicants' assertion that changes in mRNA level generally lead to corresponding changes in protein level.

The Examiner further asserts that "Orntoft et al. concentrated on regions of chromosomes with strong gains of chromosomal material containing clusters of genes... This analysis was not done for PRO1303 in the instant specification, and so it is not clear whether or not PRO1303 is in a gene cluster in a region of a chromosome that is highly amplified. Therefore, the relevance, if any of Orntoft et al. is not clear." (Pages 22-23 of the instant Final Office Action).

Applicants fail to see how these considerations are relevant to the analysis. Orntoft *et al.* did not limit their findings to only those regions of amplified gene clusters. Further, as discussed in Applicants' previous Responses, Hyman *et al.* and Pollack *et al.*, made of record November 13, 2006, did gene-by-gene analysis across all chromosomes.

Applicants respectfully submit that the Examiner also appears to misunderstand the data presented by Hyman et al. The Examiner asserts that "of the 12,000 transcripts analyzed, a set of 270 was identified in which overexpression was attributable to gene amplification." The Examiner concludes that "[t]his proportion is 2%; the Examiner maintains that 2% does not provide a reasonable expectation that the slight amplification of PRO1303 would be correlated with elevated levels of mRNA." (Page 19 of the instant Final Office Action).

Applicants respectfully submit that the Examiner appears to have misinterpreted the results of Hyman *et al.* Hyman *et al.* chose to do a genome-wide analysis of a large number of genes, most of which, as shown in Figure 2, were not amplified. Accordingly, the 2% number is meaningless, as the low figure mainly results from the fact that only a small percentage of genes are amplified in the first place. The significant figure is not the percentage of genes in the genome that show amplification, but the percentage of amplified genes that demonstrate increased mRNA and protein expression.

The Examiner further asserts that the Hyman reference "found 44% of highly amplified genes showing overexpression at the mRNA level, and 10.5% of highly overexpressed genes being amplified; thus, even at the level of high amplification and high overexpression, the two do not correlate." (Page 19 of the instant Final Office Action).

Applicants submit that the 10.5% figure is not relevant to the issue at hand. One of skill in the art would understand that there can be more than one cause of overexpression. The issue is not whether overexpression is always, or even typically caused by gene amplification, but rather, whether gene amplification typically leads to overexpression.

The Examiner's assertion is not consistent with the interpretation Hyman *et al.* themselves place on their data, stating that, "The results illustrate **a considerable influence of copy number on gene expression patterns**." (page 6242. col. 1; emphasis added). In the more detailed discussion of their results, Hyman *et al.* teach that "[u]p to 44% of the highly amplified transcripts (CGH ratio, >2.5) were overexpressed (***i.e.*, belonged to the global upper 7% of expression ratios**) compared with only 6% for genes with normal copy number." (See page 6242, col. 1; emphasis added). These details make it clear that Hyman *et al.* set a highly restrictive standard for considering a gene to be overexpressed; yet almost half of all highly amplified transcripts met even this highly restrictive standard. Therefore, the analysis performed by Hyman *et al.* clearly shows that it is "more likely than not" that a gene which is amplified in tumor cells will have increased gene expression.

The Examiner asserts that Hyman et al. and Pollack et al. do not examine protein expression. (Pages 19-20 of the instant Final Office Action).

Applicants submit that the articles by Orntoft *et al.*, Hyman *et al.*, and Pollack *et al.* were submitted primarily as evidence that in general, gene amplification increases mRNA expression. As evidence that, in general, there is a correlation between mRNA levels and polypeptide levels, Applicants further submitted the Declaration of Dr. Paul Polakis. Thus Applicants do not rely upon the Orntoft *et al.*, Hyman *et al.*, and Pollack *et al.* articles to show a correlation between mRNA levels and polypeptide levels, because such a correlation is demonstrated in the Polakis Declaration. Nonetheless, as discussed above, Orntoft *et al.* does provide evidence that increased mRNA levels in tumor cells are associated with increased protein levels in the same tumor cells.

Second Polakis Declaration

Applicants present a second Declaration by Dr. Polakis (Polakis II) that presents evidentiary data in Exhibit B. As previously disclosed in the Response to Office Action of November 13, 2006, Exhibit B of the Polakis II Declaration identifies 28 gene transcripts out of 31 gene transcripts (i.e., greater than 90%) that showed good correlation between tumor mRNA and tumor protein levels. As Dr. Polakis' Declaration (Polakis II) says "[a]s such, in the cases where we have been able to quantitatively measure both (i) mRNA and (ii) protein levels in both (i) tumor tissue and (ii) normal tissue, we have observed that in the vast majority of cases, there is a very strong correlation between increases in mRNA expression and increases in the level of protein encoded by that mRNA." Accordingly, Dr. Polakis has provided the facts to enable the Examiner to draw independent conclusions.

Applicants further submit that, as discussed in their previous Responses, the standard for utility is more likely than not. Dr. Polakis' Declarations provide evidence, in the form of statements by an expert in the art, that "an increased level of mRNA in a tumor cell relative to a normal cell typically correlates to a similar increase in abundance of the encoded protein in the tumor cell relative to the normal cell." The PRO1303 gene was found to be amplified in lung and colon tumors. As discussed above and in Applicants' previous Responses, one of ordinary skill in the art would therefore expect the PRO1303 mRNA to be overexpressed in the same human lung and colon tumor samples. Accordingly, one of ordinary skill in the art would understand that the PRO1303 polypeptide would be expected (more likely than not) to be overexpressed in human lung and colon tumor samples relative to their normal human tissue counterparts, as are the majority of other molecules tested.

Applicants reiterate that the law is clear that the Examiner must establish that it is **more likely than not** that one of ordinary skill in the art would doubt the truth of the statement of utility. The data in Exhibit B of the Polakis II Declaration shows that, in more than **90%** of their observations they have found that increases in the level of a particular mRNA correlates with changes in the level of protein expressed from that mRNA. This has far exceeded the "more likely than not" standard. Therefore, the data of Exhibit B, which indicates the probability of the correlation between mRNA and protein for a given gene, is closely related to the asserted utility of PRO1303.

Scott Declaration

Applicants have also previously submitted, with their Response filed November 13, 2006, a Declaration by Dr. Randy Scott ("the Scott Declaration"). Dr. Scott was a co-founder of Incyte Pharmaceuticals, Inc., the world's first genomic information business, and is currently the Chairman and Chief Executive Officer of Genomic Health, Inc., a life sciences company located in Redwood City, California, which provides individualized information on the likelihood of disease recurrence and response to certain types of therapy using gene expression profiling. Based on his more than 15 years of personal experience with the DNA microarray technique and its various uses in the diagnostic and therapeutic fields, and his familiarity with the relevant art, Dr. Scott unequivocally confirms that, as a general rule, there is a good correlation between mRNA and protein levels in a particular tissue.

As stated in paragraph 8 of the Scott Declaration:

> DNA microarray analysis has been extensively used in drug development and in diagnosis of various diseases. ..... Due to its importance in drug discovery and in the field of diagnostics, microarray technology has not only become a laboratory mainstay but also created a world-wide market of over $600 million in the year of 2005. A long line of companies, including Incyte, Affymetrix, Agilent, Applied Biosystems, and Amersham Biosciences, made microarray technology a core of their business.

In paragraph 10 of his Declaration, Dr. Scott explains the reasons for the wide-spread use and impressive commercial success of this technique, stating:

> One reason for the success and wide-spread use of the DNA microarray technique, which has led to the emergence of a new industry, is that generally there is a good correlation between mRNA levels determined by microarray analysis and expression levels of the translated protein. Although there are some exceptions on an individual gene basis, **it has been a consensus in the scientific community that elevated mRNA levels are good predictors of increased abundance of the corresponding translated proteins in a particular tissue**. Therefore, diagnostic markers and drug candidates can be readily and efficiently screened and identified using this technique, without the need to directly measure individual protein expression levels. (emphasis added).

The Declaration, which is based on Dr. Scott's unparalleled experience with both the microarray technique and its industrial and clinical applications, supports Applicants' position that microarray technology is not only mature, reliable and well-accepted in the art, but also has been extensively used in drug development and in diagnosis of various diseases and produced enormous commercial success. Therefore, if a gene, such as the gene encoding the PRO1303

polypeptide, has been identified to be over-expressed in a certain disease, such as lung cancer, it is more likely than not that the protein product is also overexpressed in the disease.

The case law has clearly established that in considering affidavit evidence, the Examiner must consider all of the evidence of record anew.[1] "After evidence or argument is submitted by the applicant in response, patentability is determined on the totality of the record, by a preponderance of the evidence with due consideration to persuasiveness of argument."[2] Furthermore, the Federal Court of Appeals held in *In re Alton*, "We are aware of no reason why opinion evidence relating to a fact issue should not be considered by an Examiner."[3] Applicants also respectfully draw the Examiner's attention to the Utility Examination Guidelines[4] which state, "Office personnel must accept an opinion from a qualified expert that is based upon relevant facts whose accuracy is not being questioned; it is improper to disregard the opinion solely because of a disagreement over the significance or meaning of the facts offered." The statement in question from an expert in the field (the Scott Declaration) states: "elevated mRNA levels are good predictors of increased abundance of the corresponding translated proteins in a particular tissue." Therefore, barring evidence to the contrary regarding the above statement in the Scott Declaration, this rejection is improper under both the case law and the Utility guidelines. As discussed in detail above, the various articles cited in the Office Action do not provide such countervailing evidence.

Alberts and Lewin

In response to the submitted textbook excerpts by Alberts and Lewin, the Examiner acknowledges that the teachings of Alberts and Lewin support that the initiation of transcription is the most common point for a cell to regulate gene expression. The Examiner asserts, however, that the initiation of transcription "is not the only means of regulating gene expression" according to the teaching of Alberts. (Page 21 of the instant Final Office Action).

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[1] *In re Rinehart,* 531 F.2d 1084, 189 U.S.P.Q. 143 (C.C.P.A. 1976); *In re Piasecki,* 745 F2d. 1015, 226 U.S.P.Q. 881 (Fed. Cir. 1985).

[2] *In re Alton,* 37 U.S.P.Q.2d 1578, 1584 (Fed. Cir 1996) (quoting *In re Oetiker,* 977 F.2d 1443, 1445, 24 U.S.P.Q.2d 1443, 1444 (Fed. Cir. 1992)).

[3] *Id.* at 1583.

[4] Part IIB, 66 Fed. Reg. 1098 (2001).

Applicants respectfully submit that the utility standard is not **absolute certainty**. Rather, to overcome the presumption of truth that an assertion of utility by an applicant enjoys, the PTO must establish that it is **more likely than not** that one of ordinary skill in the art would doubt the truth of the statement of utility. Therefore, Applicants **do not need** to establish that transcription initiation is **the only means** of regulating gene expression in order to meet the utility standard. Instead, as long as it is the most common point of regulation, as admitted by the Examiner, it would be more likely than not that a change in the transcription level of a gene gives rise to a change in translation level of a gene. Applicants note that both Alberts and Lewin make clear that it is far more likely than not that protein levels for any given gene are regulated at the transcriptional level. Alberts, for example, states that of all the possible points for regulating protein expression, "[f]or most genes transcriptional controls are paramount." Cell 4th at 379 (emphasis added). In a similar vein, Lewin states that "having acknowledged that control of gene expression can occur at multiple stages, and that production of RNA cannot inevitably be equated with production of protein, it is clear that the overwhelming majority of regulatory events occur at the initiation of transcription." *Genes VI* at 847-848 (emphasis added). Thus, the utility standard is met.

Meric *et al.*

With respect to Applicants' arguments regarding Meric et al., the Examiner asserts that Meric teaches that "gene expression is quite complicated, and is also regulated at the level of mRNA stability, mRNA translation, and protein stability." (Page 21 of the instant Final Office Action).

Applicants respectfully submit that Meric simply summarizes the translational regulation of cancer cells. Meric indicates that translation initiation is regulated in response to nutrient availability and mitogenic stimulation and is coupled to cell cycle progression and cell growth. Meric further discusses how alterations in translation control occur in cancer. For example, variant mRNA sequences can alter the translational efficiency of individual mRNA molecules. (see Abstract). Meric further teaches that the changes in translational efficiency of a mRNA transcript depend on the mutation of a specific mRNA sequence. (Page 973, column 2 to page 974, column 1). Meric never suggests that the translation of a cancer gene is suppressed in cancer in general, and that therefore, increased mRNA levels will not, in general, yield increased

protein levels. To the contrary, Meric teaches that the translation efficiency of a number of cancer genes is enhanced in cancer cells compared to their normal counterparts. For instance, in patients with multiple myeloma, a C-T mutation in the c-myc IRES was identified and found to cause an enhanced initiation of translation (page 974, column 1). Therefore the level of proteins encoded by these genes increases in cancer cells at an even higher magnitude than the corresponding mRNA level. Thus Meric clearly supports Applicants' assertions that it is more likely than not that, in general, changes in mRNA levels are correlated with changes in protein levels.

With respect to the over one hundred additional references cited in Applicants' Preliminary Amendment filed November 13, 2006, the Examiner asserts that "[w]ith the exception of Futcher et al., all of Applicant's newly cited references are directed to the analysis of single genes, or a small group of genes, and therefore do not demonstrate trends found across proteins in general." (Page 22 of the instant Final Office Action).

Applicants note that the submitted references, which represent experiments conducted by a large number of different research groups, demonstrate a trend of correlation found across proteins in general, and that this trend is confirmed by an overwhelming number of experiments by different researchers, using diverse experimental designs, testing various types of tissues, under numerous biological conditions. Although only a single gene or a small group of genes was tested by each individual study group, the cumulative evidence generated by over one hundred study groups certainly establishes that it is well-accepted in the art that a general mRNA/protein correlation exists.

Futcher *et al.*

With respect to Futcher et al., who did study a extensive number of genes across the entire yeast genome, the Examiner asserts that Futcher's conclusions apply only to relatively abundant proteins, and that Futcher "also admits that Gygi et al. performed a similar study and generated similar data, but reached a different conclusion." (Page 23 of the instant Final Office Action).

As discussed previously in the Response of November 13, 2006 in the section concerning Gygi *et al.*, Futcher et al. convincingly demonstrated that the different conclusions of Gygi *et al.* were due to deficiencies in the data analysis and collection techniques used by Gygi *et al.*

Newly cited references

Celis *et al.*

The Examiner cites Celis et al. to the effect that "the number of mRNA copies does not necessarily reflect the number of functional protein molecules." (Pages 21-22 of the instant Final Office Action).

Applicants respectfully submit that, in their discussion of DNA microarrays and proteomics applied to the same samples, Celis *et al.* cite Orntoft *et al.*, and note that "**in most cases there was a good correlation between transcript and protein levels**" (page 13, col. 1; emphasis added). Celis *et al.* further explain that those few cases which showed apparent discrepancies may have been due to other causes, such as post-transcriptional processing or degradation of the protein, or the choice of methods used to assess protein expression levels. Celis *et al.* also note that the observation that there is often more change in mRNAs as compared to the proteins may be due to the fact that current technologies detect mainly high abundance proteins, while most of the changes affecting protein levels may involve low abundance proteins. Thus the correlation between mRNA and protein levels may be even higher than typically observed, given these factors.

Applicants further submit that significant correlations between gene and protein expression are most likely to be observed for genes associated with cancer, since as Celis *et al.* note, "transformation resulted in the abnormal expression of normal genes, rather than in the expression of new ones" (page 11, col. 1). Accordingly, alterations in gene amplification or expression are more likely to be associated with altered protein expression in the case of cancer than in other cases where DNA microarrays are used, because, as explained by Celis *et al.*, the alterations in expression levels of certain normal proteins are part of the process that leads to cancer.

Nagaraja *et al.*, Waghray *et al.*, and Sagynaliev *et al.*

In support of the assertion that "mRNA levels are not necessarily predictive of protein levels" (page 23 of the instant Final Office Action), the Examiner cites three new references, by Nagaraja et al., Waghray et al., and Sagynaliev et al.

The Examiner cites Nagaraja et al. as allegedly teaching that in comparisons of expression profiles for normal breast compared to breast cancer, "the proteomic profiles

indicated altered abundance of fewer proteins as compared to transcript profiles." (Pages 23-24 of the instant Final Office Action).

Applicants respectfully submit that the fact that many more transcripts than proteins were found to be differentially expressed does not mean that most mRNA changes did not result in correlating protein changes, but merely reflects the fact that expression levels were only measured at all for many fewer proteins than transcripts. In particular, the total number of proteins whose expression levels could be visualized on silver-stained gels was only about 300 (page 2332, col. 1), as compared to the approximately 14,500 genes on the microarray chips for which mRNA levels were measured (page 2336, col. 1). Since the expression levels of so many fewer proteins than transcripts were measured, it is hardly surprising that a smaller absolute number of proteins than mRNAs were found to be overexpressed, because the protein products of most of the overexpressed mRNAs would not have been among the small number of proteins identified on the gels.

The Examiner next cites Waghray et al., to the effect that "for most of the proteins identified, there was no appreciable concordant change at the RNA level." (Page 24 of the instant Final Office Action).

Applicants emphasize that Applicants are asserting that a measurable change in mRNA level generally leads to a corresponding change in the level of protein expression, not that changes in protein level can be used to predict changes in mRNA level. Waghray *et al.* did not take genes which showed significant mRNA changes and check the corresponding protein levels. Instead, the authors looked at a small and unrepresentative number of proteins, and checked the corresponding mRNA levels. Waghray *et al.* acknowledge that only "[a] relatively small set of genes could be analyzed at the protein level, largely due to the limited sensitivity of 2-D PAGE" (page 1337, col. 1). In particular, while the authors examined the expression levels of16,570 genes (page 1329, col. 2), they were able to measure the expression levels of only 1031 proteins (page 1333, col. 2). Waghray *et al.* does not teach that changes in mRNA expression were not correlated with changes in expression of the corresponding protein. All Waghray *et al.* state is that "for most of the proteins identified, there was no appreciable concordant change at the mRNA level" (page 1337, col. 2). This statement is not relevant to Applicants' assertion of utility, since Applicants are not asserting that changes in mRNA levels are the only cause of

changes in protein levels. Waghray *et al.* do not contradict Applicants' assertion that changes in mRNA expression, in general, correspond to changes in expression of the corresponding protein.

Lastly, the Examiner cites Sagynaliev et al., as allegedly teaching that "it is also difficult to reproduce transcriptomics results with proteomics tools." In particular, the Examiner notes that according to Sagynaliev et al., of 982 genes found to be differentially expressed in human CRC, only 177 (18%) have been confirmed using proteomics technologies. (Page 25 of the instant Final Office Action).

The Sagynaliev *et al.* reference, titled "Web-based data warehouse on gene expression in human colorectal cancer" (emphasis added), drew conclusions based upon a literature survey of gene expression data published in human CRC, and not from experimental data. While a literature survey can be a useful tool to assist researchers, the results may greatly over-represent or under-represent certain genes, and thus the conclusions may not be generally applicable. In particular, Applicants note that, as evidenced by Nagaraja *et al.* and Waghray *et al.*, discussed above, the number of mRNAs examined in transcriptomics studies is typically much larger than the number of proteins examined in corresponding proteomics studies, due to the difficulties in detecting and resolving more than a small minority of all expressed proteins on 2D gels. Thus the fact that only 18% of all genes found to be differentially expressed in human CRC have been confirmed using proteomics technologies does not mean that the corresponding proteins are not also differentially expressed, but is most likely due to the fact that the corresponding proteins were not identified on 2D gels, and thus their expression levels remain unknown.

The authors of Sagynaliev *et al.* acknowledge the many technical problems in finding proteomic data for CRC that can be matched to transcriptomic data to see if the two correlate. The authors state that "results have been obtained using heterogeneous samples in particular cell lines, whole tissue biopsies, and epithelial cells purified from surgical speciements." However, "Results obtained in cell lines do not allow accurate comparison between normal and cancer cells, and the presence/absence of proteins of interest has to be confirmed in biopsies." (Page 3072, left column.) In particular, the authors specifically note that "only a single study [1] provided differential display protein expression data obtained in the human patient, using whole tissue biopsy." (Page 3068, left column, second paragraph; *see also*, Table 2.) The examiner also notes and the authors state, "For CRC, there is no publication comparing mRNA and protein expression for a cohort of genes." (Page 3077, left column, last paragraph, emphasis added.)

Applicants further note that Table 2 shows that 6 out of 8 published proteomics studies were done using 2-D PAGE. However, the authors state that "2-D PAGE or 2-D DIGE have well-known technological limitations ... even under well-defined experimental conditions, 2-D PAGE parallel analysis of paired CRC samples is hampered by a significant variability." (Page 3077, left column, third paragraph.) Therefore, Applicants respectfully submit that it is well known in the art that there are problems associated with selecting only those proteins detectable by 2D gels.

Lilley *et al.*, Wildsmith *et al.* and King *et al.*

*The Examiner next asserts that "the state of the art, as evidenced through textbooks and review papers, clearly establishes that polypeptide levels cannot be accurately predicted from mRNA levels." (Page 25 of the instant Final Office Action). In support of this assertion, the Examiner cites textbook excerpts by Lilley et al. and Wildsmith et al., and an article by King et al. In particular, the Examiner cites Lilley et al. to the effect that "the extrapolation that changes in transcript level will also result in corresponding changes in protein amount or activity cannot **always** be made." (Page 25 of the instant Final Office Action, emphasis added). The Examiner cites Wildsmith et al. to the effect that "the gene expression data obtained from a microarray **may differ** from protein expression data." (Pages 25-26 of the instant Final Office Action, emphasis added). Finally, the Examiner cites King et al. to the effect that "it has been established that mRNA levels do not **necessarily** correlate with protein levels." (Page 26 of the instant Final Office Action, emphasis added).*

Applicants reiterate that the evidentiary standard to be used throughout *ex parte* examination of a patent application is a preponderance of the totality of the evidence under consideration. Accordingly, in order to overcome the presumption of truth that an assertion of utility by the applicant enjoys, the Examiner must establish that **it is more likely than not** that one of ordinary skill in the art would doubt the truth of the statement of utility. **The standard is not absolute certainty.** The law requires only that one skilled in the art should accept that such a correlation is **more likely than not to exist**. The law does not require a "necessary" correlation between mRNA and protein levels. Nor is it required that protein levels can be "accurately predicted" from mRNA levels. Nowhere in these papers do the authors suggest that it is more likely than not that altered mRNA levels do not correlate with altered protein levels. On the contrary, statements such as "the extrapolation that changes in transcript level will also

result in corresponding changes in protein amount or activity cannot *always* be made" imply that the mRNA/protein correlation exits in most cases.

Applicants further note that the cited papers disclose a number of successful examples of microarray applications in human disease study, which further validate Applicants' assertions. For example, Wildsmith *et al.* points out that

> one area of rapid progress using microarray technology is the increased understanding of cancer. Molecular pathologies are subgrouping cancers of tissues such as blood, skin, and breast, based on differential gene expression patterns. For example, within a small group of breast cancer tissue samples, Perou *et al.* distinguished two broad subgroups representing those expressing or alternatively lacking expression of the oestrogen receptor- α- gene. The work was not conclusive, but never has progress in this field been so rapid when compared with the previous methods of gene amplification. Another example of the impact of this technology is in the identification of two biomarkers for prostate cancer, namely hepsin and PIM1 (Dhanasekaran *et al.*, 2001). Microarray technology has also accelerated the understanding of the molecular events surrounding pulmonary fibrosis. Specially, two distinct clusters of genes associated with inflammation and fibrosis have been identified in a disease where, for years, the pathogenesis and treatment have remained unknown (Katsuma *et al.*, 2001). (Page 284).

King *et al.* disclose that microarray technology offers tremendous advantagies in human disease study. For example, the authors state that "microarrays can be expected to prove extremely valuable as tools for the study of the generic basis of complex diseases. The ability to measure expression profiles across entire genomes provides a level of information not previously attainable...Microarrays make it possible to investigate differential gene expression in normal vs. diseased tissue, in treated vs. non-treated tissue, and in different stages during the natural course of the disease, all on a genomic scale. Gene expression profiles may help to unlock the molecular basis of phenotype, response to treatment, and heterogeneity of disease." (Page 2287, column 3).

Bork *et al.*

The Examiner also refers to a paper by Bork *et al.* (Page 26 of the instant Final Office Action). Bork *et al.* comments generally about high-throughput technologies (which include microarrays) and in fact, validates the positive potential of such technologies by admitting that such technologies "often reveal important general trends that are impossible to realize with classical, low-throughput experimental methods, yet provide fewer insights into specific,

molecular detail (see page 1, column 1, line 3-8 of the Bork article). This article comments on the limitations in the "total knowledge base" of protein function. Bork further quotes Anderson *et al.*'s coefficient of 0.48 as the correlation between mRNA and protein expression. Applicants note that a 0.48 correlation value (about 50%) supports the contention that it is "more likely than not" that protein expression correlates well with mRNA expression. Therefore, Bork supports the Applicants' position that changes in mRNA levels are generally correlated with changes in protein levels.

Madoz Gurpide *et al.*

The Examiner cites Madoz Gurpide et al. to the effect that "[f]or most of the published studies, it is unclear how well RNA levels reported correlate with protein levels." (Page 26 of the instant Final Office Action).

Applicants respectfully point out that Madoz Gurpide *et al.* state only that is it "unclear" how well RNA levels reported correlate with protein levels, not that the levels do not correlate. Madoz Gurpide *et al.* also acknowledge that DNA microarray studies "**justify the use of this technology for uncovering patterns of gene expression that are clinically informative**" (page 53; emphasis added).

Applicants respectfully submit that while proteomics is indeed a complementary technology to DNA microarrays, this does not mean that proteomic experiments are required in addition to measurements of mRNA levels to determine protein expression. The cited papers make clear that proteomic techniques are useful to obtain information beyond expression levels, such as the protein's activation state, posttranslational modifications, and subcellular localization. For example, Madoz-Gurpide *et al.* explain that mRNA expression alone does not provide information regarding "activation state, post-translational modification or localization of corresponding proteins" (page 168, col. 1). Haynes *et al.*, as quoted in the instant Final Office Action, states that "only the direct analysis of mature protein products can reveal their correct identities, their relevant state of modification and/or association, and their amounts." (Page 12 of the instant Final Office Action). Celis *et al.* note that "proteomics addresses problems that cannot be approached by DNA analysis, namely, relative abundance of the protein product, post-translational modification, subcellular localization, turnover, interaction with other proteins as well as functional aspects" (page 6, col. 2).

While this additional information may be useful in elucidating the detailed biological function of a protein, **it is not required to establish utility of a protein as a marker for cancer**, because the claimed PRO1303 polypeptides can be used in cancer diagnosis without any knowledge regarding the function or cellular role of the polypeptides. Applicants submit that the law clearly states that "it is not a requirement of patentability that an inventor correctly set forth, or even know, how or why the invention works." *Newman v. Quigg*, 11 U.S.P.Q.2d 1340 (Fed. Cir. 1989). Accordingly, the disclosure or identification of the mechanism by which PRO1303 is associated with cancer is not required in order to establish the patentable utility of the claimed PRO1303 polypeptides. Thus while Madoz-Gurpide *et al.* note that it is "more difficult to develop an understanding of disease at a mechanistic level using DNA microarrays," (page 53) this is not relevant to Applicants' assertions of utility, since, as discussed above, it is not necessary to understand how or why an invention works in order to demonstrate utility.

The Patent Office has failed to meet its initial burden of proof that Applicant's claims of utility are not substantial or credible. The arguments presented by the Examiner in combination with the Pennica, Konopka, Chen, Hanna, Hu, LaBaer, Haynes, Gygi, Lian, Fessler, Celis, Nagaraja, Waghray, Sagynaliev, Lilley, Wildsmith, King, Bork, and Madoz-Gurpide papers, do not provide sufficient reasons to doubt the statements by Applicants that PRO1303 has utility. As previously discussed, the law does not require the existence of a "necessary" correlation between mRNA and protein levels. Nor does the law require that protein levels be "accurately predicted." According to the authors themselves, the data in the above cited references confirm that there is a general trend between protein expression and transcript levels, which meets the "more likely than not standard" and show that a positive correlation exists between mRNA and protein. Therefore, Applicants submit that the Examiner's reasoning is based on a misrepresentation of the scientific data presented in the above cited reference and application of an improper, heightened legal standard. In fact, contrary to what the Examiner contends, the art indicates that, if a gene is overexpressed in cancer, it is more likely than not that the encoded protein will also be expressed at an elevated level.

Based on the above arguments, Applicants have clearly demonstrated a credible, specific and substantial asserted utility for the claimed PRO1303 polypeptides, for example, as diagnostic markers for lung tumors. Further, based on this utility and the disclosure in the specification,

one skilled in the art at the time the application was filed would know how to use the claimed polypeptides.

Applicants therefore respectfully request withdrawal of the rejections of Claims 28-35 and 38-47 under 35 U.S.C. §101 and 35 U.S.C. §112, first paragraph.

II. Claim Rejections Under 35 U.S.C. §112, First Paragraph (Scope of Enablement)

Claims 28-32 and 39-47 are newly rejected under 35 U.S.C. §112, first paragraph, as allegedly lacking enablement for the recited polypeptide variants of PRO1303.

Applicants respectfully maintain the position that that Claims 28-32 and 39-47 satisfy the enablement requirement under 35 U.S.C. §112, first paragraph, for the reasons previously set forth in Applicants' Responses filed on November 13, 2006 and the Appeal Brief filed April 24, 2006.

The Examiner further asserts that "the claims as drafted include huge numbers of possible embodiments, in which each amino acid may be altered to any of the possible 23 amino acids." (Page 31 of the instant Final Office Action).

Applicants respectfully submit that the claims do not recite all possible variants having at least 80% amino acid sequence identity to SEQ ID NO:194, but only those variants wherein the nucleic acid encoding said polypeptide is amplified in lung or colon tumor cells. Thus it is not required that Applicants demonstrate that all variants having at least 80% identity to SEQ ID NO:194 are "necessarily" amplified in lung or colon tumor cells, as the claims are limited to only those variants which are in fact amplified. As discussed in detail in their previous Responses, Example 143 provides ample information for determining whether a gene is significantly amplified, thus allowing the skilled artisan to readily determine those variants encompassed by the claims.

The Examiner also alleges that "the recitation of 'the nucleic acid encoding the polypeotide is amplified in lung and colon tumors' is not a function of the polypeptide." (Page 31 of the instant Final Office Action).

Applicants submit that the law is clear that "[a] functional limitation is an attempt to define something by what it does, rather than by what it is (e.g., as evidenced by its specific structure or specific ingredients)."[5] "A functional limitation is often used in association with an

[5] *In re Swinehart*, 439 F.2d 210, 169 U.S.P.Q. 226 (C.C.P.A. 1971).

element, ingredient, or step of a process to define a particular capability or purpose that is served by the recited element, ingredient or step."[6] Accordingly, overexpression of the claimed polypeptides in lung and breast tumor cells is a functional limitation which indicates the functional purpose (i.e., use in the diagnosis of cancer) of the claimed polypeptides.

Withdrawal of this rejection is respectfully requested.

III. Claim Rejections Under 35 U.S.C. §112, First Paragraph (Written Description)

Claims 28-32 and 39-47 remain rejected under 35 U.S.C. §112, first paragraph, as allegedly lacking adequate written description for the recited variant polypeptides of PRO1303.

Applicants respectfully maintain the position that that Claims 28-32 and 39-47 satisfy the written description requirement under 35 U.S.C. §112, first paragraph, for the reasons previously set forth above and in Applicants' Responses filed on November 13, 2006 and the Appeal Brief filed April 24, 2006.

[6] M.P.E.P. 2173.05(g).

CONCLUSION

In conclusion, the present application is believed to be in *prima facie* condition for allowance, and an early action to that effect is respectfully solicited. Should there be any further issues outstanding, the Examiner is invited to contact the undersigned agent at the telephone number shown below.

Please charge any additional fees, including fees for additional extension of time, or credit overpayment to Deposit Account No. **08-1641** (referencing Attorney's Docket No. **39780-2830 P1C14**).

Respectfully submitted,

Date: October 4, 2007

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